

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problems Mailbox.**

STIC-LL

From:
Sent:
To:
Subject:

Canella, Karen
Tuesday, March 05, 2002 6:31 PM
STIC-ILL
ill order 09/819,193

Art Unit 1642 Location 8E12(mail)

Telephone Number 308-8362

Application Number 09/819,193

1. Cancer Research:
1981 May, 41(5):1978-1983
1988 Apr 1, 48(7):1864-1873
2. Anticancer Research:
1987 Jul-Aug, 7(4B):781-789
1997, Vol. 17, No. 3C, pp. 1973-1983
3. Gynecologic Oncology:
1990 May, 37(2):188-199
1992 Jun, 45(3):273-278
1991, Vol. 42, No. 1, pp. 39-43
1982, Vol. 13, No. 2, pp. 50-57
4. Pathobiology:
1997 Jul-Aug, 65(4):177-183
1993, 61(2):67-76
1992, 60(1):33-41
5. Jikeikai Med J:
1989, 36(4):303-316
1994, 41(4):407-415
6. Sapporo Med J:
1988, 57(6):603-612
1996, 65(5):433-444
7. Acta Obstetricia et Gynecologica Scandinavica, 1995, 74(5):330-335
8. Journal of Medicine, 1997, 28(3-4):175-190
9. Experimental and Molecular Pathology, 2000 Dec, 69(3):175-191
10. International Journal of Cancer, 15 Mar 2001, 91(6):778-782
11. Experimental Cell Research, 1992 Aug, 201(2):262-272

Thanks!

AL261-A1 I57

386307

6060095

Scientific and Technical
Information Center

MAR 07 RECD

PAT. & T.M. OFFICE

11275979

CORRELATION BETWEEN THYMIDINE PHOSPHORYLASE EXPRESSION AND INVASION PHENOTYPE IN CERVICAL CARCINOMA CELLS

Masatsugu UEDA,* Yoshito TERAJ, Koji KUMAGAI, Ken UEKI, Masanori KANEMURA and Minoru UEKI

Department of Obstetrics and Gynecology, Osaka Medical College, Osaka, Japan

The correlation between thymidine phosphorylase (dThdPase) expression and invasion phenotype in human uterine cervical carcinoma cells was investigated using 10 cervical carcinoma cell lines. Semi-quantitative reverse transcription-polymerase chain reaction analysis was performed to investigate the mRNA levels of dThdPase and matrix metalloproteinase (MMP)-2 with β -actin coamplified as an internal standard. dThdPase protein expression levels were detected by highly sensitive enzyme-linked immunosorbent assay. Tumor cell migration along a gradient of substratum-bound fibronectin and invasion into reconstituted basement membrane were evaluated by haptotactic migration and invasion assay. Although dThdPase mRNA and protein expression levels differed remarkably among the cell lines, there was a statistical correlation between them ($r = 0.743$, $p = 0.0139$). dThdPase gene and protein expression levels were well correlated with the number of cells that migrated and invaded ($p < 0.05$). Moreover, there was a close correlation between MMP-2 gene and dThdPase gene and protein expression levels ($p < 0.05$). Tumor cells that produce dThdPase may have a higher invasive and metastatic potential because of their capacity to pass through tissue barriers.

© 2001 Wiley-Liss, Inc.

Key words: thymidine phosphorylase; invasion; cervical carcinoma

Tumor angiogenesis is essential for tumor development. Prevascular tumors may remain dormant *in situ* for months to years, and the switching from a subgroup of prevascular tumor cells to an angiogenic phenotype allows rapid growth, progression and metastasis.¹ Angiogenesis is induced by factors produced by the tumor or the nonmalignant cells that infiltrate the tumor. Various peptide growth factors such as vascular endothelial growth factor (VEGF),² basic fibroblast growth factor (bFGF)³ and transforming growth factor- α (TGF- α)⁴ have been found to stimulate the proliferation and motility of endothelial cells, thus inducing new blood vessel formation.

A recent study has demonstrated that thymidine phosphorylase (dThdPase) is almost identical to platelet-derived endothelial cell growth factor (PD-ECGF).⁵ Haraguchi et al.⁶ reported that dThdPase stimulates the chemotaxis of endothelial cells and possibly other cells and thus indirectly stimulates angiogenesis. Several workers have found that the expression of dThdPase shows a significant correlation with tumor angiogenesis.^{7–10} Very recently, we also reported that dThdPase expression was closely related to intratumoral microvessel density (IMVD), myometrial invasion, pelvic lymph node metastasis and patient outcome in cervical carcinomas.¹¹ Moreover, we previously demonstrated that intraarterial infusion chemotherapy induced apoptotic cell death and might prevent micrometastases in locally advanced cervical carcinomas through the inhibition of tumor angiogenesis and dThdPase expression of tumor cells.¹² Thus, dThdPase expression may be strongly linked to the process of tumor invasion and metastasis. However, there have been very few reports on the direct correlation between dThdPase expression and invasive activity in tumor cells. In this study, we investigated mRNA and protein expression levels of dThdPase in cervical carcinoma cell lines and correlated them with invasion phenotype of the cells.

MATERIAL AND METHODS

Cell culture

Experiments were conducted using six human cervical squamous cell carcinoma cell lines (SKG-I, SKG-II, SKG-IIIa, SKG-

IIIb, OMC-1 and YUMOTO) and four adenocarcinoma cell lines (HOKUG, NUZ, OMC-4 and CAC-1). The OMC-1¹³ and OMC-4¹⁴ cell lines were established in our laboratory. The SKG-I,¹⁵ SKG-II,¹⁶ SKG-IIIa and SKG-IIIb¹⁷ cell lines were kindly provided by Dr. Shiro Nozawa, Keio University, Tokyo. The YUMOTO¹⁸ and NUZ¹⁹ cell lines were kindly provided by Dr. Naotake Tanaka, Chiba University, Chiba. The HOKUG²⁰ and CAC-1²¹ cell lines were provided by Dr. Isamu Ishiwata, Ishiwata Hospital, Mito, and Dr. Osamu Hayakawa, Sapporo Medical College, Sapporo, respectively. All of the 10 cell lines, except for the YUMOTO and NUZ cell lines, were maintained as monolayer cultures in Ham's F-12 medium (Flow Laboratories, Irvine, Scotland) supplemented with 10% fetal bovine serum (Mitsubishi, Tokyo, Japan) at 37°C in a humidified incubator with 5% CO₂ in air. The YUMOTO and NUZ cells were cultured in RPMI-1640 medium (GIBCO BRL, Bethesda, MD) supplemented with 10% fetal bovine serum as described above. The cells were grown to confluence in 75-cm² tissue culture flasks (Nunc, Roskilde, Denmark), washed with PBS and then harvested after a brief treatment with 0.1% trypsin solution containing 0.02% EDTA (Flow). The cell viability was determined by Trypan blue dye exclusion prior to use.

RNA isolation and cDNA preparation

RNA was extracted from the cells by an SV-total RNA isolation kit (Promega, Madison, WI) according to the supplier's protocol. Contaminating residual genomic DNA was removed by digestion with RNase-free DNase (Promega). cDNAs were prepared using at least 2 μ g of total RNA and SUPERScript II reverse transcriptase (GIBCO BRL Life Technologies, Gaithersburg, MD) with random hexamers as primers and were finally dissolved in diethyl pyrocarbonate-treated water at a concentration of 1 μ g/ μ l and frozen at -20°C until use.

Reverse transcription-polymerase chain reaction analysis

Oligonucleotide primers for reverse transcription (RT)-PCR were designed using a published sequence of dThdPase, matrix metalloproteinase (MMP)-2 and β -actin genes and synthesized by the solid-phase triester method. The primers used in this study and the expected sizes from the reported cDNA sequence are shown in Table I. For PCR, cDNA aliquots were diluted in sterile water, depending on transcript abundance. Five cDNA concentrations for dThdPase/ β -actin or MMP-2/ β -actin primer combinations were used. For accurate quantification using this method, measurements must be taken in the linear phase of the reaction, in which cDNA concentration is directly proportional to signal intensity. A range of cDNA concentrations was used to determine the linear phase of the PCR. cDNA template was amplified by PCR in a final volume of 20 μ l reaction containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM MgCl₂, 0.01% (w/v) gelatin, 200 μ M dNTP, 0.5 μ M each primer and 1.25 units Taq polymerase (Perkin-Elmer, Nor-

*Correspondence to: Department of Obstetrics and Gynecology, Osaka Medical College, 2-7 Daigakumachi, Takatsuki, Osaka 569-8686, Japan. E-mail: gyn017@poh.osaka-med.ac.jp

Received 17 July 2000; Revised 15 September 2000; Accepted 16 October 2000

Published online 22 January 2001

TABLE 1-PRIMERS FOR dThdPase, MMP-2, AND β -ACTIN AMPLIFICATION

Gene	Primer	Tm (°C)	Product size (bp)
dThdPase	5'-ACAAGGTCAGCCTGGTCCTC-3'	64	344
	5'-TCCGAACCTTAACGTCCACCAC-3'		
MMP-2	5'-CCAGGTGACAAGCCCATGGGGCCCC-3'	58	480
	5'-GCAGCCTAGCCAGTCGGATTGTATG-3'		
β -Actin	5'-CTTCTACAATGAGCTGCGTG-3'	60	305
	5'-TCATGAGGTAGTCAGTCAGC-3'		

walk, CT). After an initial denaturation at 96°C for 3 min, 35 cycles of denaturation (94°C for 1 min), annealing (64°C for dThdPase, 58°C for MMP-2 and 60°C for β -actin for 1 min) and extension (72°C for 2 min) for the respective target genes were carried out on a Perkin-Elmer GeneAmp PCR System 9700. The final extension was performed at 72°C for 10 min. The number of cycles in the RT-PCR was determined so as to obtain logarithmic amplification of each gene for semi-quantitative analysis of the expression levels of the genes. After visualization of the RT-PCR products by 1.5% agarose gel electrophoresis with ethidium bromide staining, gel images were obtained using the ATTO densitograph UV-image analyzer (ATTO, Tokyo, Japan), and the densities of the products were quantified using the ATTO densitometry software, version 2. The relative expression levels were calculated as the density of the product of dThdPase divided by that of β -actin from the same cDNA.

dThdPase assay

dThdPase expression of tumor cells was assayed according to the method reported by Nishida *et al.*²² with some modifications. Tumor cells in a logarithmic growth phase were harvested, washed twice with PBS and collected by centrifugation. The pellet was homogenized in a 10-fold volume of 10 mM Tris-HCl buffer (pH 7.4) containing 15 mM NaCl, 1.5 mM MgCl₂, and 50 μ M potassium phosphate and then centrifuged at 10,000 g for 15 min. The supernatant was stored at -80°C until use. The protein concentration of the supernatant was determined by using a DC protein Assay Kit (Bio-Rad, Hercules, CA). A 96-well microtiter plate (Nunc) was incubated at 4°C overnight with 10 μ g/ml of the dThdPase monoclonal antibody (MoAb) 104B in 10 mM PBS (pH 7.6). The plate coated with the MoAb was then incubated with 3% (w/v) skim milk in PBS (blocking buffer) for 1 hr at room temperature. The plate was washed with PBS containing 0.05% Tween 20 and 0.05% sodium azide and kept at 4°C until use. The blocking buffer containing 0.5% EDTA was added to all wells. Test samples and standard solutions of recombinant dThdPase, which is serially diluted with a blocking buffer, were dispensed onto the plate coated with the antibody. The plate was incubated at 37°C for 2 hr, washed with 0.05% Tween 20 in PBS and then incubated with biotinylated dThdPase MoAb 232-2 at 2 μ g/ml in blocking buffer for 1 hr at 37°C. The plate was then washed and incubated with 5,000-fold diluted 1 mg/ml streptavidin conjugated with horseradish peroxidase (Prozyme, San Leandro, CA), which was diluted with washing buffer containing 1 mg/ml bovine serum albumin, for 1 hr at room temperature. After washing, the plate was incubated with a substrate solution containing 0.4 mg/ml 3,3',5,5'-tetramethylbenzidine and 0.02% hydrogen peroxide for 10 to 20 min at room temperature. The peroxidase reaction was stopped by the addition of 1 M phosphate solution, and the amount of dThdPase sandwiched with the two anti-dThdPase MoAbs was estimated by measuring its absorbance at 450 nm with a plate reader (Bio-Rad, model 3550). The amount of dThdPase was calibrated with that measured for the standard solutions.

Haptotactic migration assay

Tumor cell migration along a gradient of substratum-bound fibronectin was assayed in Chemotaxicell culture chambers (Kurabo, Osaka) according to the methods reported by McCarthy and Furcht²³ with some modifications as previously described.²⁴ Polyvinylpyrrolidone-free polycarbonate filters with 8.0 μ m pore

size were precoated with 10 μ g of fibronectin in a volume of 50 μ l of PBS on the lower surface and dried overnight at room temperature under a hood. Log-phase cell cultures of tumor cells were harvested with 0.1% trypsin containing 0.02% EDTA and resuspended to a final concentration of 3.0×10^6 /ml in growth medium. The cell suspension (200 μ l) was added to the upper compartment, and 600 μ l of growth medium was immediately added to the lower compartment. The chambers were then incubated for 24 hr at 37°C in a 5% CO₂ air. The filters were fixed with ethanol and stained with hematoxylin. The cells on the upper surface of the filters were removed by wiping with a cotton swab. The cells that had migrated to various areas of the lower surface were manually counted under a microscope at a magnification of $\times 400$. Each assay was performed in triplicate.

Invasion assay

The invasive activity of tumor cells was assayed according to the method reported by Albini *et al.*²⁵ with some modifications as previously described.²⁴ Briefly, the lower surface of the filters was precoated with fibronectin as described above. Matrigel, diluted to 500 μ g/ml with cold PBS, was applied to the upper surface of the filters (5 μ g/filter). The following procedures were the same as those of the haptotactic migration assay. Each assay was performed in triplicate.

Statistical analysis

The Spearman rank correlation coefficient was used to analyze the relation between two different values. A level of $p < 0.05$ was accepted as statistically significant.

RESULTS

As shown in Figure 1a, expression of dThdPase mRNA in 10 human cervical carcinoma cell lines explored by our RT-PCR analysis differed remarkably among the cells. The relative gene expression levels of dThdPase in comparison with β -actin expression ranged widely from 0.001 in OMC-4 cells to 0.226 in YU-MOTO cells. The protein expression levels of dThdPase detected by highly sensitive enzyme-linked immunosorbent assay also ranged widely, from 0.3 in OMC-4 cells to 825.3 ng/mg protein in SKG-II cells. There was a statistically significant correlation between dThdPase gene and protein expression levels, with a coefficient correlation of 0.743 ($p = 0.0139$), as shown in Figure 1b.

We next examined whether mRNA and protein expression levels of dThdPase in cervical carcinoma cell lines are correlated with the invasion phenotype of the cells using haptotactic migration and invasion assay. As can be seen in Figure 2, there was a statistically significant correlation between dThdPase gene expression and the number of tumor cells that migrated and invaded, with coefficient correlations of 0.707 and 0.766 ($p = 0.0222$ and 0.0098), respectively. Moreover, the protein expression levels of dThdPase were also well correlated with the number of tumor cells that migrated and invaded, with coefficient correlations of 0.734 and 0.846 ($p = 0.0156$ and 0.0020), respectively, as shown in Figure 3.

Since proteinase expression is closely related to invasion phenotype of tumor cells, we finally examined MMP-2 gene expression in 10 cervical carcinoma cell lines by RT-PCR analysis. The relative gene expression levels of MMP-2 in comparison with β -actin expression ranged from 0.044 in NUZ cells to 0.314 in

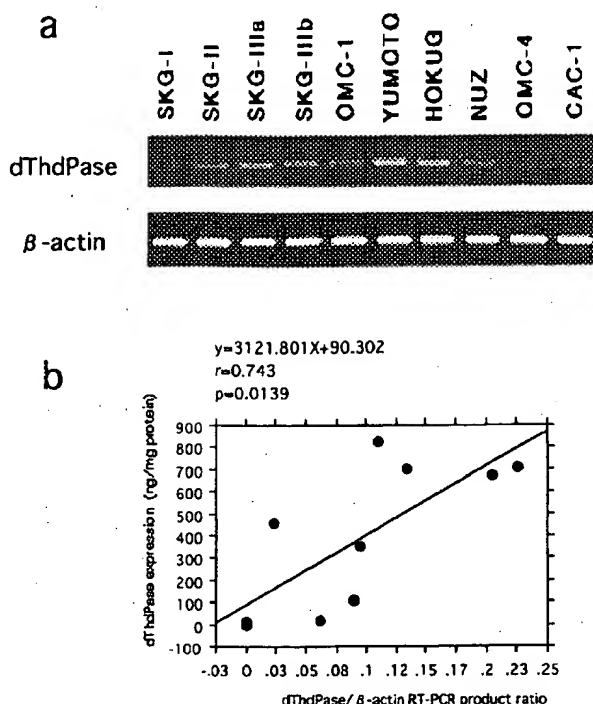


FIGURE 1 – RT-PCR analysis of dThdPase in 10 human cervical carcinoma cell lines (a) and correlation between dThdPase gene and protein expression levels (b). The primers and the expected sizes from the reported cDNA sequence are shown in Table I. Plots show the correlation between dThdPase/ β -actin RT-PCR product ratio and dThdPase protein expression (ng/mg protein).

YUMOTO cells. As shown in Figure 4, there was a close correlation between MMP-2 gene and dThdPase gene and protein expression levels, with coefficient correlations of 0.710 and 0.635 ($p = 0.0213$ and 0.0487), respectively.

DISCUSSION

Growth of solid tumors depends on angiogenesis, the process by which new blood vessels develop from the endothelium of a pre-existing vasculature. Abundant evidence supports the concept that tumors can induce angiogenesis through a variety of angiogenic molecules, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), TGF- α and dThdPase/PD-ECGF, released from the tumor cells and/or tumor-associated inflammatory cells.²⁻⁶ Among several potential angiogenic factors, expression of dThdPase has previously been demonstrated in breast,⁷ bladder,²⁶ gastric,^{8,27,28} colorectal,²⁹ endometrial,¹⁰ and ovarian⁹ carcinomas. We recently reported that dThdPase expression in invasive cervical carcinomas was closely associated with IMVD, tumor invasion, pelvic lymph node metastasis and overall survival.¹¹ Recent studies have also found that immunohistochemical expression of dThdPase is correlated with tumor vascularity,⁷⁻¹⁰ depth of tumor invasion,²⁹ lymph node metastasis^{28,29} and hematogenous metastasis.^{8,27} These previous reports suggest that dThdPase expression may be closely related to tumor invasion and metastasis.

Metastatic spread of the solid tumor depends on a critical cascade of events that includes tumor cell adhesion to a distant site, extracellular matrix degradation, migration, proliferation and, ultimately, neovascularization.³⁰ The primary tumor with a high proportion of angiogenic cells is likely to give rise to metastatic implants that are already angiogenic, enabling them to grow in

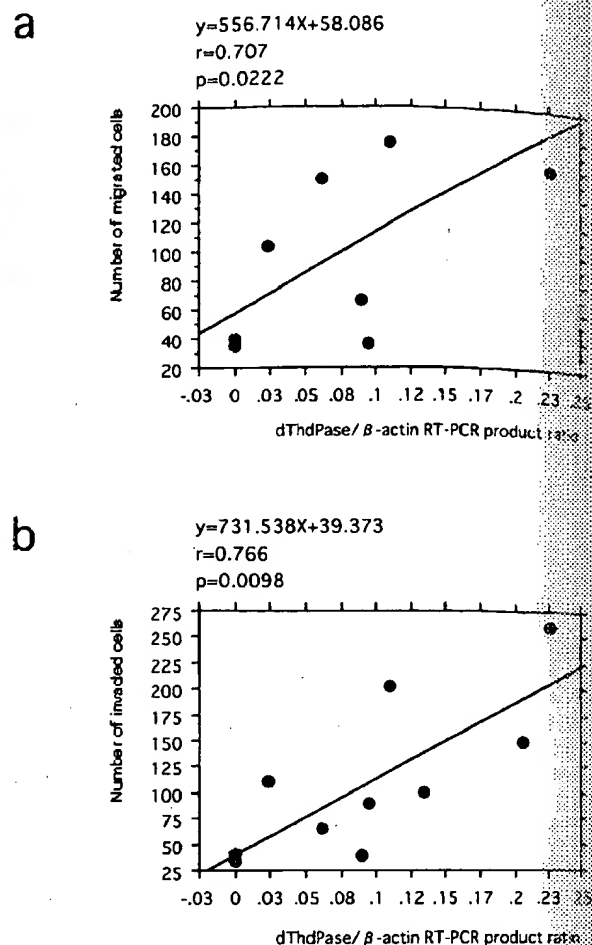


FIGURE 2 – Correlation between dThdPase/ β -actin RT-PCR product ratio and the number of cells that migrated (a) and invaded (b). Each point represents the mean of triplicates.

lymph nodes and distant organs.³¹ In other words, tumors that produce a higher level of angiogenic factors may display more aggressive behavior than tumors negative for those factors in the process of invasion and metastasis.

In the present study, we revealed that dThdPase gene and protein expression levels in cervical carcinoma cell lines were closely related to the number of cells that migrated and invaded. Nishida *et al.*²² previously demonstrated that dThdPase activity levels were well correlated with dThdPase expression levels in a variety of tumor cells. Moreover, there was a close correlation between MMP-2 gene and dThdPase gene and protein expression levels in cervical carcinoma cell lines examined. MMP-2 (72 kD type IV collagenase), one of the proteolytic enzymes, is closely linked to the invasion phenotype of tumor cells.^{24,32} Therefore, cervical carcinoma cells that produce dThdPase may have a higher invasive and metastatic potential because of their capacity to pass through tissue barriers. Ishikawa *et al.*³³ demonstrated that expression of type IV collagenase and of dThdPase was simultaneously up-regulated by the cytokines in Lewis lung carcinoma cells. We also reported that epidermal growth factor (EGF) and TGF- α stimulated functional activity or expression of both proteinases and dThdPase in cervical and ovarian carcinoma cells and that these growth factors promoted the *in vitro* invasive activity of the cells.^{24,32} It would be of interest to investigate the signaling path-

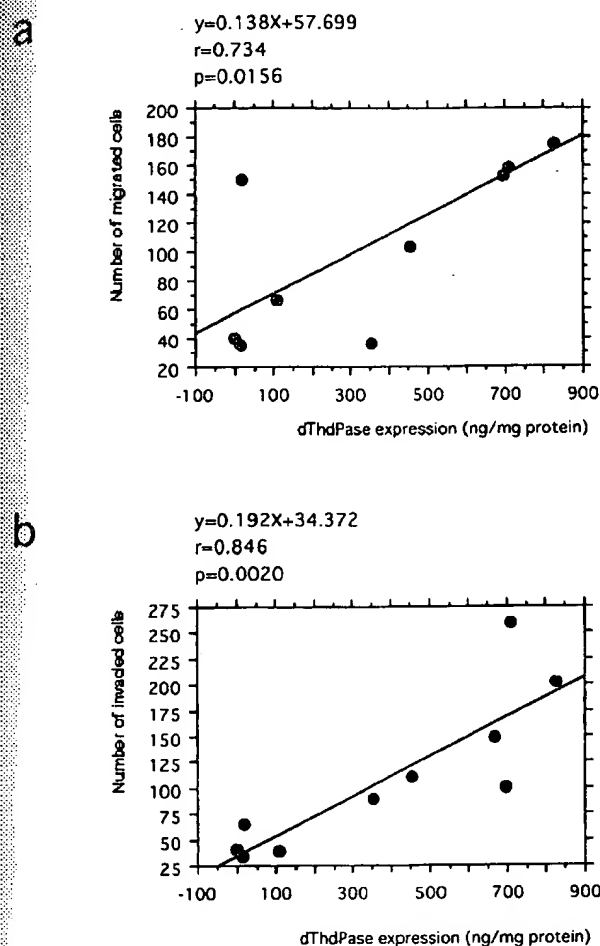


FIGURE 3—Correlation between dThdPase protein expression and the number of cells that migrated (a) and invaded (b). Each point represents the mean of triplicates.

ecules and transcriptional factors that regulate both proteinases and dThdPase gene expression.

To the best of our knowledge, this is the first report to demonstrate a direct correlation between dThdPase expression and the invasion phenotype of tumor cells, which may provide a partial explanation for the clinical evidence given above. However, tumor cell invasion is a complex multistage process involving cell attachment, migration and the degradation of tissue barriers caused by various proteolytic enzymes secreted by tumor cells. Further studies are needed to clarify the molecular events leading to tumor

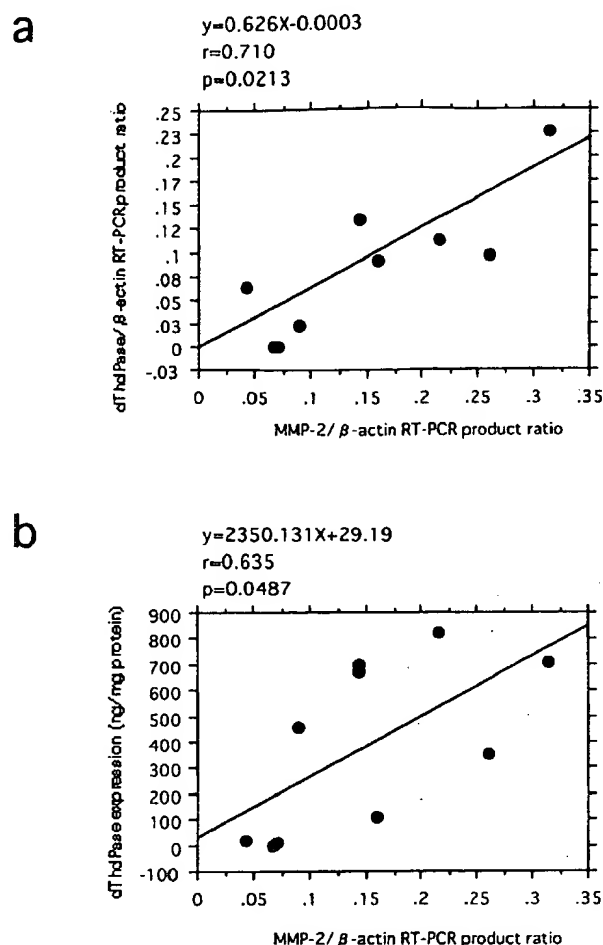


FIGURE 4—Correlation between MMP-2/β-actin RT-PCR product ratio and dThdPase/β-actin RT-PCR product ratio (a) or dThdPase protein expression (b). Each point represents the mean of triplicates.

cells with a higher invasive activity induced by dThdPase, using dThdPase-transfected tumor cells.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. S. Nozawa, Keio University, Tokyo, Dr. N. Tanaka, Chiba University, Chiba, Dr. I. Ishiwata, Ishiwata Hospital, Mito and Dr. O. Hayakawa, Sapporo Medical College, Sapporo, for the gift of cervical carcinoma cell lines. We also thank Nippon Roche Co. and Kyowa Hakko Co. for their advice and interest and K. Sato for her technical assistance.

REFERENCES

- Folkman J. Clinical applications of research on angiogenesis. *N Engl J Med* 1995; 333: 1757-63.
- Connolly, DT. Vascular permeability factor: a unique regulator of blood vessel function. *J Cell Biochem* 1991; 47:219-23.
- Ohtani H, Nakamura S, Watanabe Y, Mizoi T, Saku T, Nagura H. Immunocytochemical localization of basic fibroblast growth factor in carcinomas and inflammatory lesions of the human digestive tract. *Lab Invest* 1993; 68: 520-7.
- Malden, LT, Novak U, Burgess AW. Expression of transforming growth factor alpha messenger RNA in the normal and neoplastic gastro-intestinal tract. *Int J Cancer* 1989; 43: 380-4.
- Furukawa T, Yoshimura A, Sumizawa T, Haraguchi M, Akiyama S. Angiogenic factor. *Nature (Lond)* 1992; 356: 668.
- Haraguchi M, Miyadera K, Uemura K, Sumizawa T, Furukawa T, Yamada K, Akiyama S. Angiogenic activity of enzymes. *Nature (Lond)* 1994; 368: 198.
- Toi M, Hoshina S, Taniguchi T, Yamamoto Y, Ishitsuka H, Tominaga T. Expression of platelet-derived endothelial cell growth factor/thymidine phosphorylase in human breast cancer. *Int J Cancer* 1995; 64: 79-82.
- Tanigawa N, Amaya H, Matsumura M, Katoh Y, Kitaoka A, Aotake T, Shimomatsuya T, Rosenwasser OA, Iki M. Tumor angiogenesis and expression of thymidine phosphorylase/platelet derived endothelial cell growth factor in human gastric carcinoma. *Cancer Lett* 1996; 108: 281-90.
- Nakanishi Y, Kodama J, Tokumo K, Seki N, Miyagi Y, Yoshinouchi M, Okuda H, Kudo T. The expression of platelet-derived endothelial cell growth factor/thymidine phosphorylase associates with angiogenesis in epithelial ovarian cancer. *Int J Clin Oncol* 1997; 2: 219-23.

10. Fujiwaki R, Hata K, Iida K, Koike M, Miyazaki K. Immunohistochemical expression of thymidine phosphorylase in human endometrial cancer. *Gynecol Oncol* 1998; 68: 247-52.
11. Ueda M, Terai Y, Kumagai K, Ueki K, Okamoto Y, Ueki M. Correlation between tumor angiogenesis and expression of thymidine phosphorylase, and patient outcome in uterine cervical carcinoma. *Hum Pathol* 1999; 30: 1389-94.
12. Ueda M, Ueki K, Kumagai K, Terai Y, Okamoto Y, Ueki M, Otsuki Y. Apoptosis and tumor angiogenesis in cervical cancer after preoperative chemotherapy. *Cancer Res* 1998; 58: 2343-6.
13. Ueda M, Ueki M, Yamada T, Okamoto Y, Maeda T, Sugimoto O, Otsuki Y. Scatchard analysis of EGF receptor and effects of EGF on growth and TA-4 production of newly established uterine cervical cancer cell line (OMC-1). *Hum Cell* 1989; 2: 401-10.
14. Ueda M, Ueki M, Sugimoto O. Characterization of epidermal growth factor (EGF) receptor and biological effect of EGF on human uterine cervical adenocarcinoma cell line OMC-4. *Hum Cell* 1993; 6: 218-25.
15. Taguchi S. Establishment and characterization of the human uterine cervical epidermoid cancer cell line. *Acta Obstet Gynecol Jpn* 1981; 33: 1180-8.
16. Ishiwata I, Nozawa S, Kiguchi K, Kurihara S, Okumura H. Establishment of human uterine cervical cancer cell line and comparative studies between normal and malignant uterine cervical cells in vitro. *Acta Obstet Gynaecol Jpn* 1978; 30: 731-8.
17. Nozawa S, Udagawa Y, Ohta H, Kurihara S, Fishman WH. Newly established uterine cervical cancer cell line (SKG-III) with Regan isoenzyme, human chorionic gonadotropin β -subunit, and pregnancy-specific β 1-glycoprotein phenotypes. *Cancer Res* 1983; 43: 1748-60.
18. Mitsuhashi A, Tanaka H, Tanaka N, Sugita M, Shirasawa H, Tokita H, Eda H. Establishment and characterization of a new HPV-negative squamous cell carcinoma cell line (Yumoto) from the human uterine cervix. *Gynecol Oncol* 1998; 70: 339-47.
19. Suzumori K, Yasui Y. NUC-1. *Hum Cell* 1988; 1: 454.
20. Ishiwata I, Ishiwata C, Soma M, Ono I, Nakaguchi T, Nozawa S, Ishikawa H. Differences between cell lines of uterine cervical glassy cell carcinoma and large cell nonkeratinizing squamous cell carcinoma. *Anal Quant Cytol Histol* 1990; 12: 290-8.
21. Koizumi M, Uede T, Shijubo N, Kudo R, Hashimoto M, Kikuchi K. New monoclonal antibody, 1C5, reactive with human cervical adenocarcinoma of the uterus, with immunodiagnostic potential. *Cancer Res* 1988; 48: 6565-72.
22. Nishida M, Hino A, Mori K, Matsumoto T, Yoshikubo T, Ishitsuka H. Preparation of anti-human thymidine phosphorylase monoclonal antibodies useful for detecting the enzyme levels in tumor tissues. *Biol Pharm Bull* 1996; 19: 1407-11.
23. McCarthy JB, Furcht LT. Laminin and fibronectin promote the haptotactic migration of B16 mouse melanoma cells in vitro. *J Cell Biol* 1984; 98: 1474-0.
24. Ueda M, Ueki M, Terai Y, Morimoto A, Fujii H, Yoshizawa T, Yanagihara T. Stimulatory effects of EGF and TGF- α on invasive activity and 5'-deoxy-5-fluorouridine sensitivity in uterine cervical carcinoma SKG-IIIb cells. *Int J Cancer* 1997; 72: 1027-33.
25. Albini A, Iwamoto Y, Kleinman HK, Martin GR, Aaronson SA, Kozlowski JM, McEwan RN. A rapid in vitro assay for quantitating the invasive potential of tumor cells. *Cancer Res* 1987; 47: 3239-45.
26. O'Brien TS, Fox SB, Dickison AJ, Turley H, Westwood M, Moghaddam A, Gatter KC, Bicknell R, Harris AL. Expression of the angiogenic factor thymidine phosphorylase/platelet-derived endothelial cell growth factor in primary bladder cancers. *Cancer Res* 1994; 56: 4799-804.
27. Maeda K, Chung YS, Ogawa S, Takatsuka S, Kang SM, Ogawa M, Sawada T, Onoda N, Kato Y, Sowa M. Thymidine phosphorylase/platelet-derived endothelial cell growth factor expression associated with hepatic metastasis in gastric carcinoma. *Br J Cancer* 1996; 73: 884-8.
28. Takebayashi Y, Miyadera K, Akiyama S, Hokita S, Yamada K, Akiba S, Yamada Y, Sumizawa T, Aikou T. Expression of thymidine phosphorylase in human gastric carcinoma. *Jpn J Cancer Res* 1996; 87: 288-95.
29. Takebayashi Y, Akiyama S, Akiba S, Yamada K, Miyadera K, Sumizawa T, Yamada Y, Murata F, Aikou T. Clinicopathologic and prognostic significance of an angiogenic factor, thymidine phosphorylase, in human colorectal carcinoma. *J Natl Cancer Inst* 1996; 88: 1110-7.
30. Liotta LA, Steeg PS, Stetler-Stevenson WG. Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. *Cell* 1991; 64: 327-36.
31. Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis—correlation in invasive breast carcinoma. *N Engl J Med* 1991; 324: 1-8.
32. Ueda M, Fujii H, Yoshizawa K, Kumagai K, Ueki K, Terai Y, Yanagihara T, Ueki M. Effects of sex steroids and growth factors on invasive activity and 5'-deoxy-5-fluorouridine sensitivity in ovarian adenocarcinoma OMC-3 cells. *Jpn J Cancer Res* 1998; 89: 1334-42.
33. Ishikawa T, Ura M, Yamamoto T, Tanaka Y, Ishitsuka H. Selective inhibition of spontaneous pulmonary metastasis of Lewis lung carcinoma by 5'-deoxy-5-fluorouridine. *Int J Cancer* 1995; 61: 516-21.